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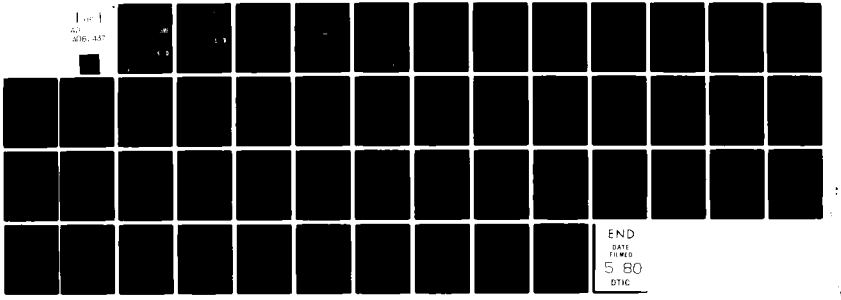

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A SUMMARY AND EVALUATION OF AQUATIC ENVIRONMENTAL DATA IN RELATION TO ESTABLISHING WATER QUALITY CRITERIA FOR MUNITIONS-UNIQUE COMPOUNDS

PART 2: NITROGLYCERIN

FINAL REPORT

J.H. SULLIVAN, JR., H.D. PUTNAM, M.A. KEIRN,
B.C. PRUITT, JR., J.C. NICHOLS, AND J.T. McCLAVE

APRIL 1979

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U.S. ARMY MEDICAL RESEARCH & DEVELOPMENT COMMAND

Ft. Detrick, Frederick, MD 21701

J. GARETH PEARSON, PROJECT OFFICER
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The purpose of this report is to review the effects of nitroglycerin (TNG) on the aquatic environment and to recommend water quality criteria for the protection of aquatic organisms.

Chemical properties, analytical methods, manufacturing wastewater characteristics, and environmental fate of TNG are reviewed and discussed.

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20. Abstract

Four species of algae were exposed to TNG concentrations up to 10 mg/l for 96 hour periods. Effects on cell counts and chlorophyll a were measured. Two organisms, Anabaena flos-aquae and Microcystis aeruginosa, were unaffected at the highest dose level. The results for Selenastrum capricornutum were invalidated by excessive response to the lactose vehicle added in conjunction with the TNG. Navicula pelliculosa showed reduced growth and chlorophyll a values at 0.32 mg/l but no effect at 0.1 mg/l.

Acute toxicity studies were conducted on three species of macroinvertebrates and one zooplankton species. EC50 values for 48-hour exposure ranged from 46 to 55 mg/l for static tests and 20 to 32 mg/l for flow-through tests. Chronic studies showed effects on Daphnia magna at 12.5 mg/l but no effect at 6.2 mg/l; for Chironomus tentans, effects were found at 3.1 mg/l with no effect at 1.5 mg/l.

Four species of fish, rainbow trout, channel catfish, fathead minnow, and bluegill, were subjected to 96-hour toxicity tests. LC50 results ranged from 1.38 to 5.5 mg/l with most values between 2 and 4 mg/l. Bioconcentration tests showed little bioconcentration potential (8 to 15X). Longer term (30 day) critical life stage studies were conducted using channel catfish and fathead minnows. Full life cycle tests were conducted using fathead minnows. For catfish, the lowest significant response concentration was 0.31 mg/l with no effect at 0.15 mg/l. For fathead minnows the lowest response concentration was 0.06 mg/l with no effect at 0.03 mg/l.

Three procedures were utilized to determine the recommended water quality criteria for TNG; 1) a new proposed procedure by EPA, 2) acute toxicity values multiplied by a general application factor, and 3) acute toxicity values multiplied by an experimentally derived application factor. Considering the results of these three procedures and the data from the chronic studies, a water quality criteria for TNG of 0.01 mg/l (24-hour average) was recommended.

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PART 2: NITROGLYCERIN

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I. Introduction

Since the early part of the 1970's the U.S. Army Medical Research and Development Command has been supporting research to determine the environmental hazards associated with waste discharges of its munitions industry. The objective of these studies has been to develop the data-base required from which water quality criteria for munitions unique compounds can be established by examining effects on mammalian and aquatic species and communities. The data-base for the target compounds has been under periodic review to determine the necessary requirements for final water quality criteria. The development of these criteria has been authorized by the Federal Water Pollution Control Act, as amended by the Clean Water Act of 1977 (Public Law 92-500).

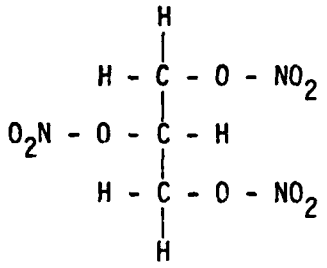
The purpose of this report is to review the effects of nitroglycerin, more properly called glyceryl trinitrate (TNG), on the aquatic environment and to recommend water quality criteria for the protection of aquatic organisms. This substance is classified as an explosive and is used commercially to make dynamite. It also is used in medicine as a vasodilator. In military munitions, TNG has been employed predominantly as a component of double and triple-based propellants combined with nitrocellulose and with nitrocellulose plus nitroguanidine, respectively. The principal source of TNG in wastewater stems from the manufacturing process; however, appreciable amounts are also present in propellant processing wastes. Chemical characterization of wastewater has shown that the less nitrated esters of glycerol, mono- and dinitrates, are also present in environmental discharges. TNG has been considered to break down sequentially to the less nitrated forms, based on the results of microbial transformations, and more importantly, animal metabolism studies (Glennon et al. 1977). The data base generated for aquatic hazard evaluation has therefore been largely restricted to TNG itself. The impact of this material has been examined by assessing vertebrate, invertebrate, and algal toxic response by conducting laboratory bioassays. In addition, field investigations have examined the response of selected aquatic communities to this substance.

The recommended acute and chronic safe levels for water were developed from the existing data base using the guidelines of the American Public Health Association (APHA) (1975), National Academy of Sciences (NAS) (1973), and Environmental Protection Agency (1976A & 1978) methodology. The latter documents contain the existing and proposed strategy necessary to provide application factors for predicting environmentally safe levels based on laboratory bioassays.

II. Chemical and Physical Properties

Glyceryl trinitrate, also called trinitroglycerol or nitroglycerin, is an explosive aliphatic nitrate ester which has been used since 1864 as a commercial blasting explosive and may be considered the first organic high explosive suitable for practical applications. TNG is a pale yellow, viscous liquid prepared by nitrating glycerol with either a mixture on concentrated nitric acid and concentrated sulfuric

acid or a mixture of concentrated plus fuming nitric acids. Completely esterified glycerol, 1,2,3-propanetriol trinitrate, has the chemical formula $C_3H_5(NO_3)_3$ and the structure shown below composed of sp^3 carbon-carbon bonds.



The theoretical nitrogen content is 18.5 percent for the trinitrated ester. Military-grade glyceryl trinitrate must contain at least 18.4 percent N (Mark et al. 1965).

Since glyceryl trinitrate is a nitrate ester, the terms nitroglycerin and trinitroglycerol are not in accord with the best chemical nomenclature. These names imply that the compounds contain "nitro" groups ($-NO_2$) generally associated with aromatic nitrated compounds. Since much of the literature has used the acronym TNG (trinitroglycerol), this report will also refer to the compound as TNG.

TNG (molecular weight 227.09) does not polymerize and exists as a stable solid at temperatures below 13.5°C in the form of dipyramidal crystals. Liquid TNG begins to become volatile and to decompose at 50-60°C. At 145°C the decomposition is so rapid that the liquid appears to boil (Mark et al. 1965). At 256°C TNG explodes spontaneously. When stored at temperatures below 50°C, it is stable for many years (Mark et al. 1965).

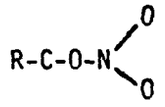
Small quantities of TNG will burn without exploding, however this compound is capable of developing a large amount of explosive energy. As measured by the "sand test" (Mark et al. 1965), the brisance value is one of the highest recorded, exceeded only by ethylene glycol dinitrate (EGDN) and PETN (pentaerythritol tetranitrate). Because of the high specific gravity of TNG, this energy can be concentrated in a relatively small volume. TNG is very unstable and sensitive to both heat, shock and concussion and thus presents a severe handling risk. The general properties of TNG are summarized in Table 1. The appreciable solubility of TNG in water, 1800 mg/l at 20°C - 2460 mg/l at 60°C, indicates that environmentally significant concentrations may be dissolved in waste rinse water and would be expected to remain in the water column.

Reactivity. Poly-nitrated alcohols as a group owe their chemical instability and brisance to the configuration of the nitrate ester bonds.

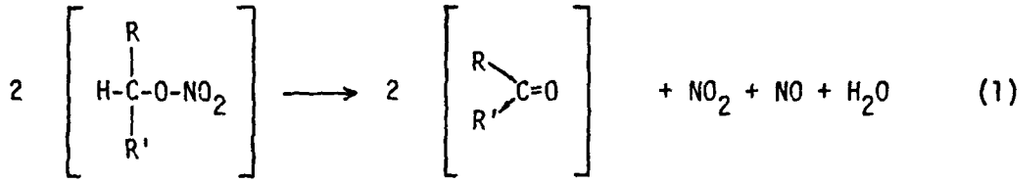
TABLE 1

GENERAL CHEMICAL AND PHYSICAL PROPERTIES OF TNG

CAS No.	55-63-0
Name:	1,2,3- Propanetriol trinitrate Glyceryl trinitrate, trinitro- glycerol, Nitroglycerin, Trinitrin, TNG
Molecular Weight:	227.09
Empirical Formula:	$C_3H_5(NO_3)_3$
Physical State:	Viscous liquid; specific gravity $1.592^{20°}_{4°}$
Color:	Pale yellow
Percent Nitrogen	18.5
Carbon	15.9
Oxygen	63.4
Hydrogen	2.2
Melting Point:	13.5°C / 2.8°C (labile form)
Boiling Point:	Apparently boils by decomposing rapidly at temperatures above 145°C. Explodes at 256°C.
Specific Heat:	1580 cal/g
Autoignition Temperature	250-260°C
Vapor Pressure	2.6×10^{-4} mm Hg @ 20°C
Solubility Characteristics:	
Water	0.18% w/v
Ethanol	25% w/w
Methanol	5.6% w/w
Carbon Disulfide	0.83% w/w
Complete Miscible in:	Benzene, Ethyl acetate, Acetone, Ether, Glacial acetic acid, Nitrobenzene, Chloroform, Toluene, Phenol, Ethylene chloride, other nitrate esters.
References:	Hawley 1977; Merck and Co. 1976; Weast 1971; Rosenblatt et al. 1973; Mark et al. 1965.

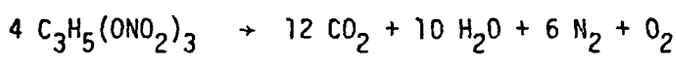


where the $O-NO_2$ moiety is coplanar but perpendicular to the carbon atom. Proximity of several nitrate groups sets up carbon-carbon and carbon-oxygen bond stresses. Explosive decomposition releases nitric oxide and nitrogen dioxide to yield an aldehyde or ketone of the parent alcohol (Boschan et al. 1955):



(1)

Very little energy input is required to initiate this reaction, however, the massive energy output from the first step generally results in complete oxidation of the organic moiety to gaseous products. In the case of TNG, the overall reaction yields nitrogen gas, carbon dioxide, water, and oxygen (Mark et al. 1965; Wibaut 1951):



(2)

This equation shows that TNG possesses an oxidizing value (Mark et al. 1965) of 105.9 percent.

The sensitivity of TNG to impact is of the same order as that of the detonating agents lead azide and mercury fulminate (Mark et al. 1965), especially at elevated temperatures. TNG can be stabilized by combining it with other materials, however. Commercial dynamite, for example, is composed of 75 percent TNG absorbed in 25 percent diatomaceous earth.

Behavior in the Aquatic Environment. Because of its appreciable solubility, a significant portion of the transformations of TNG will occur in the water column. These transformations have been thought to involve sequential chemical and biochemical hydrolysis of the nitrate ester moieties followed by metabolism of the resultant non-nitrated compounds (Dacre & Tew 1973; Glennon et al. 1977). The EPA (1976B) has reviewed TNG

waste treatment processes and stated that wastewater with up to 1200 mg/l TNG is amenable to treatment by the activated sludge process after addition of lime to reduce the explosion hazard.

Walsh (1976) and Bogatko (1978) studied biodegradation of nitrate esters in activated sludge systems. In contrast to the EPA report cited above, Walsh found that TNG concentrations greater than 75 mg/l in incoming wastewater caused a toxic response. Bogatko's results also suggest that microbial activity does not completely degrade TNG since only a 40 to 45 percent reduction in 80 mg/l concentrations of mixtures of TNG and ethylene glycol dinitrate could be attained.

Dacre and Tew (1973) reviewed the toxicology of TNG and suggested that the metabolism of this compound in mammals followed the pathway shown in Figure 1. This pathway was largely determined using radiotracer techniques. In this review, no reaction rates or evidence for aquatic microbial transformations were given. In mammals, Ellis et al. (1976A and 1976B) further documented the pathways shown in Figure 1 using thin layer chromatography and ^{14}C radiotracer methods. Radiocarbon rapidly appeared in respired air as $^{14}CO_2$, and in urine and biliary excretions as mono- and dinitroglycerides and glycuronic acids.

Characterization of TNG wastewaters and studies of the transformation products formed during microbial or chemical wastewater treatment suggest that all of the mono- and dinitrated isomers can be present as well as TNG. In addition, side reactions apparently occur which result in transformation products other than inorganic nitrites and nitrates, CO_2 and glycerol (Capellos et al. 1978; Frazer 1968; Hackley et al. 1974; and Walsh 1976).

Hackley et al. (1974) partially characterized Radford Army Ammunition Plant (RAAP) TNG wastewaters using gas-liquid chromatographic techniques (glc). Nine peaks were detected by electron capture in TNG soda wash water and 7 in less concentrated storehouse water. TNG, 1-, and 2-mononitrated glycerol (1-MNG and 2-MNG) were confirmed in both wastewater types by thin layer chromatography; the presence of the two dinitrated esters was assumed to account for two peaks in the soda wash water but not the storehouse water. Quantitation of the organic material by glc using a flame ionization detector, however, suggested that about 14 percent of the extractable material in the soda wash water was of compounds other than glycerol or its mono-, di-, and trinitrated esters. Boschan et al. (1955) attributed the production of such compounds to the occurrence of three distinct reaction mechanisms for basic and neutral hydrolysis of nitrate esters. Only one results in the formation of an alcohol. These are illustrated below:

Nucleophilic substitution:

$$OH^- + RONO_2 \rightarrow ROH + NO_3^- \qquad (3)$$

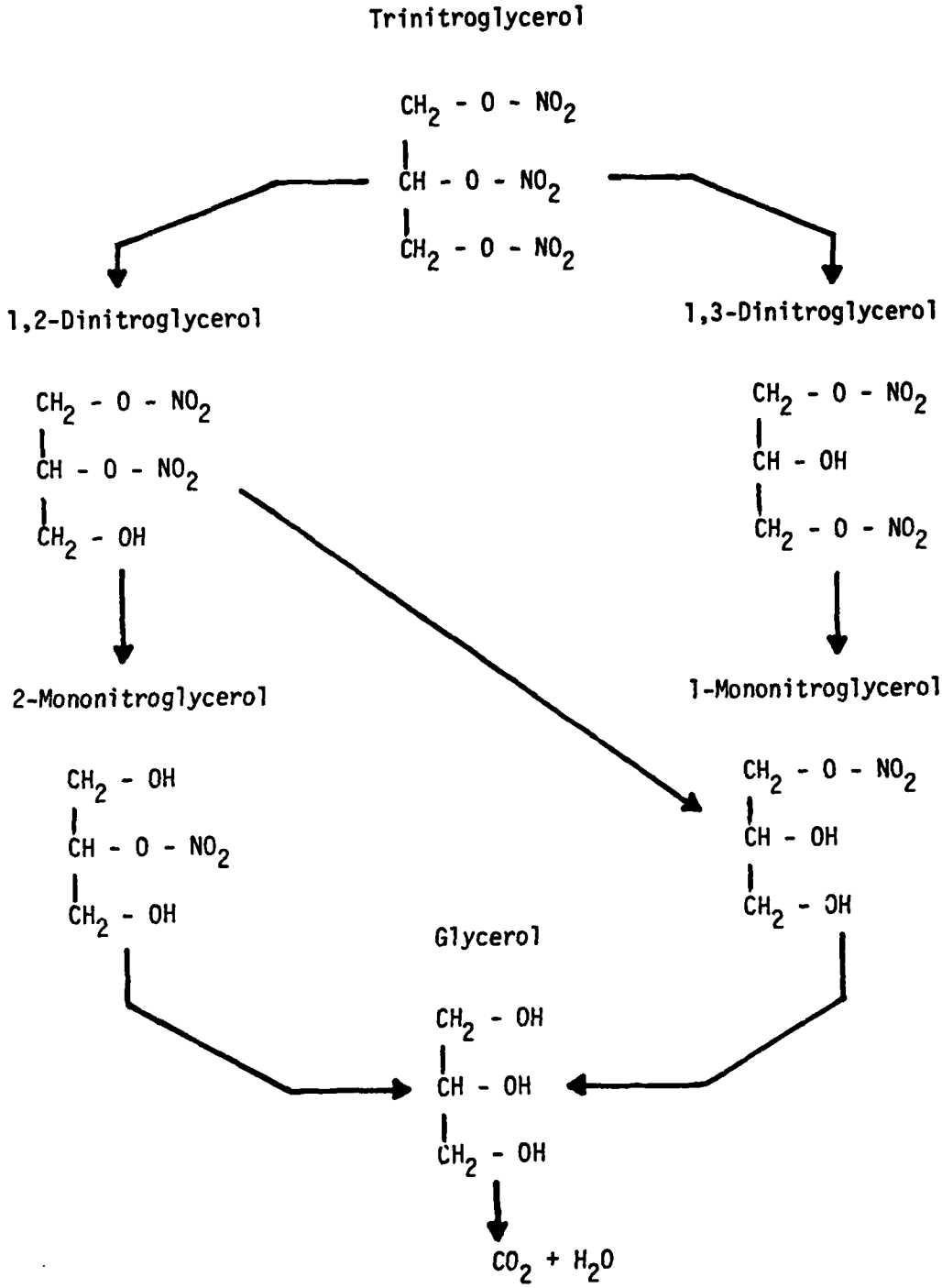
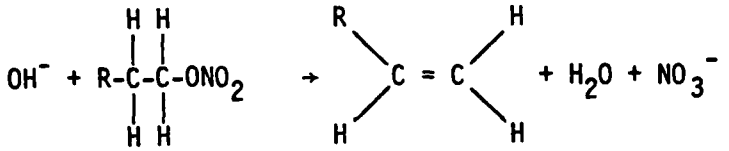


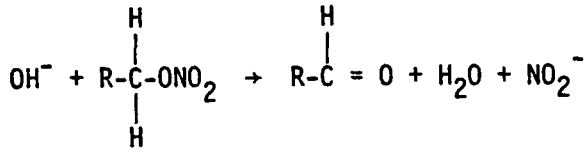
FIGURE 1. METABOLIC PATHWAYS FOR NITROGLYCERIN IN MAMMALS.
Reference: Dacre and Tew (1973)

Elimination of β-hydrogen:



(4)

Elimination of α-hydrogen:



(5)

These three reactions are bimolecular, however, hydrolysis would tend to show first order kinetics in neutral aqueous systems where H_2O is a primary reactant. Basic hydrolysis would tend to more closely approximate second order characteristics (Boschan et al. 1955).

Walsh (1976) studied TNG biodegradation by sewage bacteria using high speed liquid chromatography followed by UV detection as well as thin layer chromatography. The transformation products were partially characterized by mass spectral, infrared, and nuclear magnetic resonance techniques. Approximately 50 percent of the TNG was transformed by this system which consisted of an activated sludge reactor to which TNG was added at 70 mg/l. His results suggested that the major bacterial breakdown pathway followed sequential hydrolysis of ester linkages in a specific manner. Only one glyceryl dinitrate isomer and one glyceryl nitrate isomer were recovered. No mass balances were performed to determine if CO_2 was evolved. In addition, the analytical system would not detect glycerol. The further breakdown of these partially nitrated esters, therefore, could not be confirmed. Characterization of methylene chloride extracts of the transformation products detected the presence of compounds which had C=C bonds as well as compounds which contained bonds which represented either one or a combination of the following linkages:

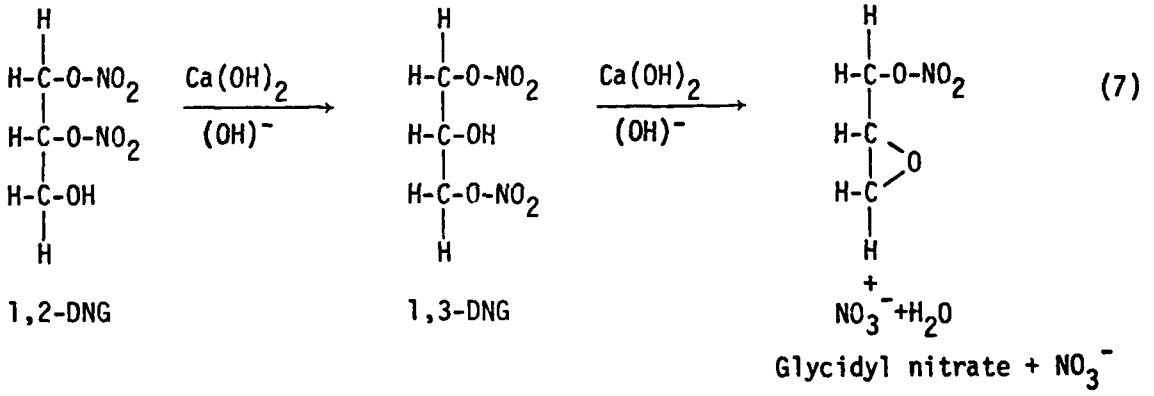
carbonyl (C=O); alcohol (C-OH); ether (C-O-C); or epoxide ().

These studies were qualitative and did not document the complete pathway. Consequently, persistence, rate of formation, and relative concentration of the various intermediate products cannot be determined. The results of Walsh (1976) suggest the following pathway for microbial hydrolysis of TNG to specific di- and mononitrated isomers (DNG and MNG):

TNG → 1,3-DNG → 2-MNG (6)

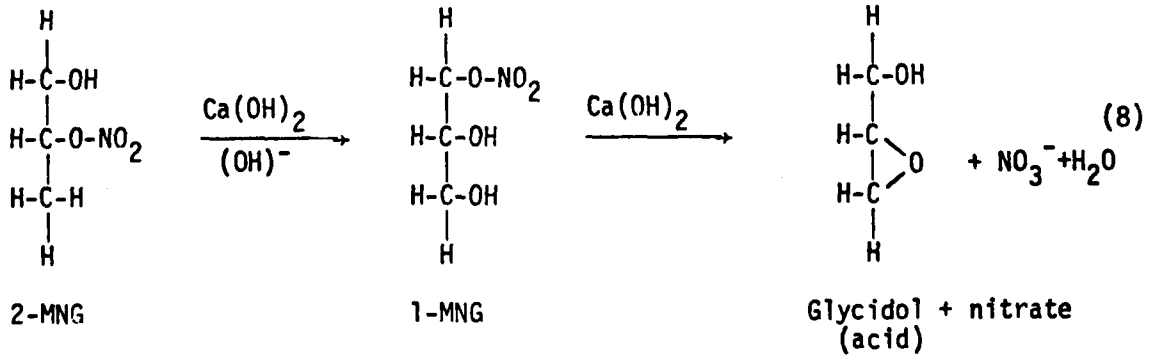
Frazer (1968) studied the hydrolysis of TNG, 1,3-DNG, and 1- and 2-MNG in ethanol solutions of NaOH and found evidence of both α and β elimination via second order reactions. The relative ease of hydrolysis was: TNG > 1,3-DNG >1-MNG > 2-MNG in order of decreasing reaction rate. Basic hydrolysis using $Ca(OH)_2$ has been proposed as a treatment method for RAAP wastewater, therefore the kinetics of basic hydrolysis of TNG and the isomeric DNG's in aqueous solution have been studied by the U.S. Army Medical Research and Development Command. This research has been conducted since July 1976, (Capellos et al. 1978), and has resulted in isolation of some of the chemical breakdown products. In general, hydrolysis reactions obeyed second order kinetics in aqueous $Ca(OH)_2$ solutions. The relative ease of hydrolysis in water was different than in alcohol. Based on the second order decay constant, the 1,3-DNG isomer was the most reactive material followed by 1,2-DNG, then TNG (Capellos et al. 1978). The reaction sequences below were proposed for the aqueous alkaline hydrolysis of glyceryl dinitrates and glyceryl nitrates:

Glyceryl dinitrates - under basic conditions, HNO_3 is eliminated from 1, 3-DNG to form an epoxy nitrate (glycidyl nitrate).



(7)

Glyceryl nitrates - under basic conditions, HNO_3 is eliminated from 1-MNG to form glycidol (1-hydroxy-2,3-epoxy propanol).



(8)

These reactions probably are the result of α elimination of the NO_3^- from the 1,3-DNG and 1-MNG. Apparently the β forms rearrange prior to hydrolysis. Glycidyl nitrate can probably also hydrolyze to glycidol (Capellos et al. 1978).

The aqueous chemical hydrolysis of TNG by hydroxide ion apparently does not follow a stepwise hydrolysis to yield DNG and MNG isomers even though both NO_3^- and NO_2^- are liberated (Capellos et al. 1978). These authors partially characterized the products of TNG hydrolysis by aqueous $Ca(OH)_2$ as polar organic compounds, nitrate esters (not MNG or DNG), calcium formate, and calcium oxalate. Other polymeric compounds which contained nitrate ester and hydroxyl groups constituted a portion of the residue. No glycidol or glycidyl nitrate was formed from hydrolysis of TNG itself.

The biochemical and chemical transformations of TNG in the aquatic environment apparently do not completely parallel enzymatic breakdown in mammalian tissue. Although stepwise hydrolysis of TNG occurs in aquatic systems, other reactions give rise to transformation products which have possible environmental significance. For example, epoxides (-C-C-) are

considered to be mutagenic and carcinogenic and have been designated high priority compounds for testing under Public Law 92-500 Section 307, and the Toxic Substances Control Act (Public Law 94-469) (Dominguez 1978). One epoxide, (glycidol) has been positively identified as a product of DNG and MNG hydrolysis. Figure 2 summarizes potential pathways of transformation of TNG in the aquatic environment. Since MNG's and DNG's normally accompany TNG in wastewater, these compounds are also included. None of the studies to date, however, documents the rate of breakdown of TNG or its less nitrated analogs in aquatic systems, nor do any data exist on the persistence of the transformation products.

Manufacturing Wastewater Characteristics. TNG is currently manufactured for the Army at RAAP, located on the New River near Radford, Virginia. The U.S. Army also controls two additional TNG manufacturing facilities which are inactive at present. One of these is Sunflower Army Ammunition Plant located in Kansas; the other is Badger Army Ammunition Plant (BAAP) located in Wisconsin. Studies of the environmental fate and effects of discharges into the receiving waters at RAAP and BAAP are described in Section IV.

The manufacturing processes at BAAP and RAAP have been summarized by Patterson et al. (1976). Glyceryl trinitrate is made by adding glycerol to a mixture of concentrated HNO_3 and H_2SO_4. Continuous agitation and cooling keep the reaction mixture at a temperature below 25°C. When the reaction is complete, the acid phase is decanted or removed and the raw TNG washed with water and then neutralized with aqueous sodium carbonate (soda wash water). Two processes are used by the U.S. Army for TNG production - the batch process which was used at BAAP and the continuous Biazzi process which is used at RAAP. The wastewaters from TNG manufacture typically contain large amounts

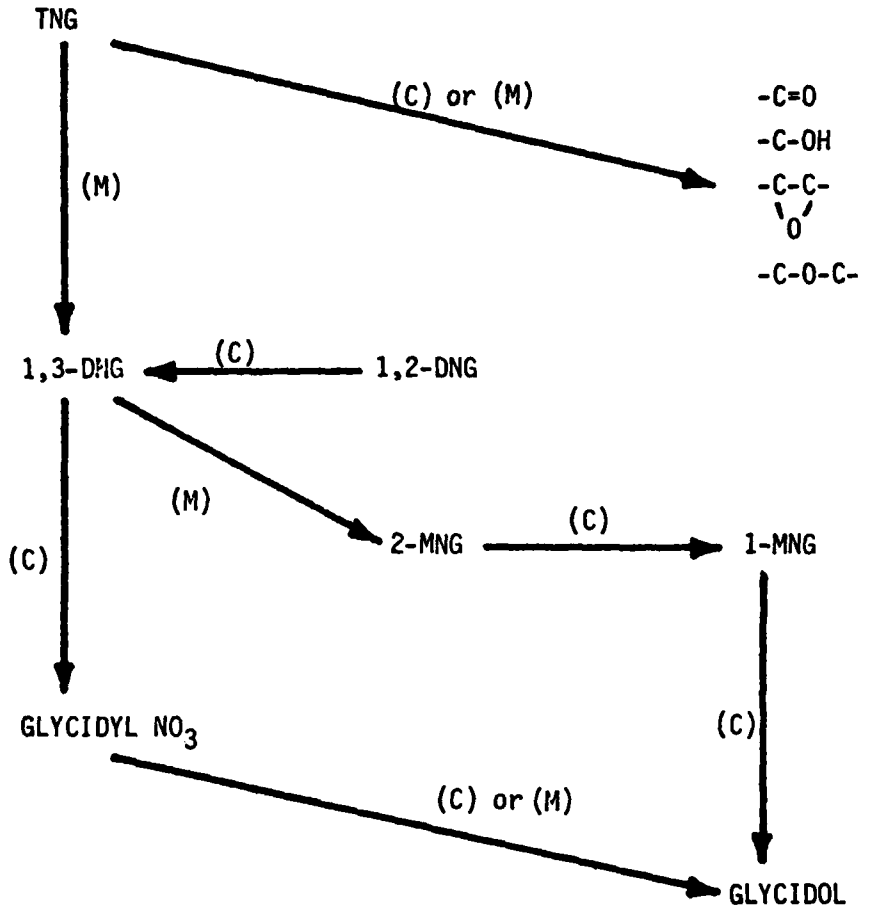


FIGURE 2. POTENTIAL BREAKDOWN PATHWAY OF TNG IN THE AQUATIC ENVIRONMENT.
(C) = chemical hydrolysis.
(M) = metabolic hydrolysis (bacteria).

of organic carbon, suspended solids, total Kjeldahl nitrogen, ammonia, nitrate, and sulfate, as well as variable quantities of the glycerol nitrates. Typical wastewater characteristics for BAAP and RAAP discharges are given in Tables 2 and 3. TNG concentrations in raw wastewater range up to the solubility limit. Traces of another compound, o-nitrodiphenylamine, also frequently appear in TNG wastewater (Weitzel et al. 1976). This material is added as a NO_3^- scavenger and stabilizer for TNG formulations. Processed TNG is stored under water at RAAP until needed for propellant manufacture. Excess water from the storage facility, contaminated with TNG, is periodically discharged into the process waste stream. Other small quantities of TNG-contaminated wastewater result from equipment wash-down operations.

Table 4 summarizes typical wastewater characteristics of discharges from the manufacture of commercial dynamite preparations. In addition to TNG and less nitrated glycerol esters, commercial wastewater contains concentrations of EGDN. In practice, commercial explosive manufacturers use up to 90 percent EGDN in dynamite formulations since the raw material, ethylene glycol, is significantly cheaper than glycerol (Bogatko 1978). EGDN also lowers the freezing point of dynamite.

At BAAP, raw wastewater combined from process, washing, and cooling water flows was discharged into two percolation ponds after neutralization. The wastewater was characterized by having low pH except when Na_2CO_3 neutralizing solutions were dumped, at which time pH fluctuated over a range from 1.7 to 9.5 units. A neutralization facility did little to alleviate this pH fluctuation. Groundwater contamination by TNG from these ponds has not been documented; however, no water supply wells exist in the vicinity of the plant.

The majority of contaminants at RAAP result from the discharges of the nitrator, i.e., the soda wash water. However, the small volume of storehouse water is also significantly contaminated. Waste treatment at RAAP has consisted of catch basins to trap sedimented and precipitated TNG. Waste streams at this plant are neutralized before collection (Patterson et al. 1976). As discussed in the previous section, proposed treatment of these waters will include $Ca(OH)_2$ hydrolysis followed by an activated sludge process employing a rotating biological contactor. Treatment of wastes high in TNG is still in the experimental stage (EPA 1976B) and reported effectiveness of biological waste treatment to completely degrade TNG remains somewhat questionable.

II.A. Analytical Methods/Environmental Monitoring

The classical methods for determining TNG in explosives and pharmaceutical preparations have been listed by Rosenblatt et al. (1973). Most of the techniques are not sensitive enough to detect this material at concentrations at which TNG becomes environmentally significant (0.01 - 10 mg/l). In general, the methods below are not suitable:

1. The Du Pont nitrometer method which measures nitric oxide gas liberated from the ester by mercury.

TABLE 2

NITROGLYCERIN WASTEWATER SOURCES AT RAAP (From Patterson et al. 1976).

Source	Flow (gpd)	COD (mg/l)	NO_3^-(N) (mg/l)	Sulfate (mg/l)	Nitroglycerin (mg/l)	Nitroglycerin (lb/day)
Nitrator	2,500	1,228	13,280	1,416	1,300	27
Air Compressor	15,000	72	3	28	Nil	Nil
Spent Acid	20,000	22	433	760	Nil	Nil
Storehouse	2,500	912	477	130	266	6

TABLE 3

TYPICAL RAW WASTEWATER QUALITY AT BAAP AND RAAP (From Patterson et al. 1976).

Parameter	RAAP Mean	RAAP Range	BAAP Mean	BAAP Range
TKN, mg N/l	< 1.6	< 0.5 - 6.0	2.54	1.1 - 5.1
NH_3-N, mg N/l	-	-	1.66	0.85 - 3.05
NO_2+NO_3-N, mg N/l	458	0.3 - 1920	117	0.5 - 200
SO_4, mg/l	145	14 - 466	243	62 - 415
COD, mg/l	< 82	< 10 - 195	109	18 - 340
TOC, mg/l	86	5 - 420	35	19 - 56
Total Solids, mg/l	8150	111 - 25,400	-	-
Suspended Solids, mg/l	< 6.4	< 1 - 39	-	-
pH	-	8.8 - 9.9	-	1.7 - 9.5
Nitroglycerin, mg/l	106	0 - 315	-	-
Flow Conditions	0.053 MGD		0.11 MGD (25% capacity)	

TABLE 4

TYPICAL RAW WASTEWATER CHARACTERISTICS FROM COMMERCIAL TNG PREPARATION (EPA 1976B)

Parameter	Mean and Concentration Range (mg/l)	
	Nitrator	Storehouse/Emulsifier
Biochemical Oxygen Demand, 5-day	8.6 (8.4-9.2)	3.2 (2.4-4.1)
Chemical Oxygen Demand	4.5 (1.5-6.5)	912 (460-1456)
Total Organic Carbon	1228 (1000-1400)	477 (200-630)
Nitrates, as N	51 (22-67)	106 (60-148)
Sulfates, as SO_4	12,380 (7500-20,000)	130 (20-179)
Suspended Solids	23.0 (3-63)	11.3 (3.3-22.1)
Dissolved Solids	81,626 (68,000-99,000)	13,905 (2952-30,848)
TNG	1300 (800-1800)	266 (83-490)
DNG	850 (520-1180)	130 (41-248)

2. Hydrolysis of the ester followed by reduction of the NO_3^- and analysis of NO_2^-.

3. Reduction by titanous chloride and back titration of the excess reagent with ferric alum.

4. Reduction with ferrous chloride and titration of the ferric iron with titanous chloride.

5. Infrared detection of nitrate groups.

6. Ferrous sulfate-sulfuric acid colorimetric method.

Two instrumental methods exist which are capable of quantitating sub-10 ppm concentrations of TNG in environmental samples. Walsh (1976) and Weitzel et al. (1976) describe high pressure liquid chromatographic separation followed by UV detection of the nitrate ester groups. Gas-liquid chromatography (glc) using electron capture has also been successfully employed (Stilwell et al. 1976 and Hackley et al. 1974). Both techniques involve solvent extraction of the TNG from water or sediment with benzene, ethyl acetate, or methylene chloride, followed by gel cleanup and concentration of the extract, if required. Because TNG is extremely thermolabile, gentle drying at ambient temperatures is required. Ultra-violet detection is not specific for TNG but choice of column and internal standards can be used to identify peaks of TNG or its less completely nitrated analogs. One advantage of ultra-violet detection is that it is done at ambient temperatures. Weitzel et al. (1976) reported detection limits of 0.002 mg/l in water and 1 mg/kg in sediments from the New River at RAAP. Walsh (1976) was able to detect as little as 0.02 mg/l (100 ppm gave a full-scale response in a 25 µl sample).

The high column and detector temperatures required for glc cause breakdown and loss of sensitivity to very low levels of TNG; however, sensitive detection is feasible if column temperatures are maintained at from 100°C to 140°C. Rosseel and Bogaert (1973) were able to detect concentrations of 0.5 µg/l in plasma using electron capture techniques. Stilwell et al. (1976) reported 100 percent recovery of TNG standards at 1 mg/l but only 28 percent recovery at 0.1 mg/l. These authors reported that detection with glc electron capture was possible to levels as low as 0.01 mg/l in water and 0.05 mg/kg in sediment but that reproducibility was poor below 0.6 mg/l and 1.5 mg/kg for water and sediment, respectively. Hackley et al. (1974) effectively used flame ionization detection as well as electron capture to quantitate TNG and related compounds at concentrations of 10-1000 mg/l.

III. Toxicological Aspects

Wastewater generated during the synthesis of TNG may contain over 1000 mg/l of this material as a dissolved component along with mono- and dinitrated esters. Propellant mixing and storage operations also produce wastewater which is contaminated with TNG. Even though TNG is at least partially biodegradable, concentrations of a few mg/l (up to 9 ppm) have been found in receiving waters, and concentrations as high as 50 mg/kg of TNG have

been found in aquatic sediments (see Section IV). An early review of the scientific literature (Dacre and Tew 1973) showed that no aquatic toxicity data were available but that TNG did have a toxic effect on mammals.

Bentley et al. (1973), under contract with the U.S. Army Medical Research and Development Command, performed an extensive laboratory evaluation of the toxicity of TNG to a wide variety of aquatic organisms representing four important trophic levels, algal primary producers, zooplankton, invertebrates, and fish. More definitive chronic studies including life cycle, reproduction, and bioconcentration were performed using selected test organisms.

With the exception of a small amount of data on the effects of TNG concentrations on activated sludge systems and one fish bioassay study of the acute toxicity of TNG and mixtures of TNG and EGDN (Hemphill 1975), the work by Bentley et al. (1978) comprises the entire data base which is available for hazard evaluation in the aquatic environment. The aquatic toxicological data base is restricted to whole organism studies and is principally related to TNG. None of the breakdown products generated during chemical or biological action on TNG have been directly evaluated. The following paragraphs provide a review of this information which elicits a variable toxic response in aquatic organisms in each of the major trophic categories.

Microorganisms. TNG generally has been considered the most labile of the principal munitions compounds (EPA 1976B; Glennon et al. 1977) based on mobility studies and on the ability of mammalian cells to metabolize it. The Atlas Powder Company (Bogatko 1978)* has carried out a series of experiments to determine the biological treatability of mixtures of TNG and ethylene glycol dinitrate. Neutralized wastewater was fed into a pilot activated sludge plant to give approximately an 80 mg/l concentration of the mixed esters in the influent. During a two-week test, this treatment method was able to reduce the nitrate ester concentration by only about 40 percent.

The incomplete biotransformation of TNG by bacteria has also been documented by Walsh (1976). In this study, concentrations of TNG above 75 mg/l were found to be toxic to the microflora of activated sludge. As a result, bacteria were exposed to an initial level of 70 mg/l. The transformation products of TNG formed by bacterial action have been discussed in Section II. The bacterial culture was able to degrade approximately 50 percent of the TNG when it was added at the 70 mg/l concentration.

These data suggest that TNG can be partially metabolized by bacteria. A toxic effect occurs above 75 mg/l, either due to a direct effect of TNG or as a response to a transformation product. Quantitative information on specific secondary toxicants is absent. Related dose-response information would be useful for further research on waste treatability.

*Bogatko, H. F. 1978. Personal communication.

Bentley et al. (1978) investigated the toxicity of TNG to four species of algae: Microcystis aeruginosa and Anabaena flos-aquae (two cyanophytes), Navicula pelliculosa (a diatom) and Selenastrum capricornutum (a chlorophyte). Procedures outlined by the EPA (Algal Assay Procedure: Bottle Test, EPA 1971) were used to define the response to triplicate challenge concentrations and controls. Chlorophyll a and either cell numbers (S. capricornutum, M. aeruginosa and N. pelliculosa) or optical density (A. flos-aquae) were measured as response parameters. TNG concentrations for these studies were not measured directly in any test vessels. The test consisted of measuring algal responses to 6 nominal TNG concentrations ranging from 0.1 mg/l to 10 mg/l. The amount of growth after 96 hours was reduced in comparison with controls grown in the absence of TNG for three of the four species. Anabaena flos-aquae was unaffected by up to 10 mg/l of TNG. A 96-hour EC50 was calculated for the mean response data of the three other species by calculating the percent inhibition relative to control culture means, converting this percentage to a probit (Finney 1971), and plotting the probit against the logarithm of nominal challenge concentration. Growth of Selenastrum capricornutum and Navicula pelliculosa was more strongly retarded than growth of either cyanophyte species. The EC50 for Microcystis aeruginosa was estimated to be greater than 10 mg/l, although this organism showed reduced growth as inhibition relative to control cultures at higher concentrations of TNG. Procaryotic microorganisms apparently are much less strongly affected than the more complex, eucaryotic algae.

Use of the probit transformation to analyze growth response relative to a control is a questionable procedure (Finney 1971) because a critical assumption which must be made is that the data to be transformed are binominally distributed. Continuous responses such as percent growth reduction or amount of growth of a culture are generally normally distributed.

According to Bentley et al. (1978), Selenastrum capricornutum showed a stimulatory response to the lactose vehicle added in conjunction with the TNG. A 10 mg/l lactose solution, equivalent to the lactose in the 1.0 mg/l TNG test assay produced 24 percent more growth, based on cell numbers at 96 hours, than control cultures. The 96-hour EC50 for this species was reported as 0.4 mg/l based on cell numbers corrected for lactose and 1.0 mg/l based on chlorophyll a analyses. This latter analysis was not corrected for lactose effects. The lactose correction was made simply by increasing the percent reduction in cell numbers for a given TNG concentration by 24 percent. No allowance was made for any lessening of this effect at the lower lactose levels found in the lower TNG concentrations. The lactose correction represented up to 10 times the percent reduction actually seen in some of the raw data. Establishment of a 96-hour EC50 for Selenastrum at 0.4 to 1.0 mg/l of TNG is therefore questionable. The 96-hour EC50 for Navicula pelliculosa was 3.3 mg/l based on cell numbers and 1.0 mg/l based on chlorophyll a as estimated by probit analysis (Bentley et al. 1978). Growth of N. pelliculosa was unaffected by lactose solutions of 10 mg/l and 100 mg/l in control tests.

The raw 96-hour chlorophyll a and 24 and 96-hour algal count data of Bentley et al. (1978) for the N. pelliculosa and the 96-hour algal counts and chlorophyll a data for the S. capricornutum were re-examined using an

analysis of variance procedure (Steel and Torrie 1960). This was done in order to establish the statistical validity of the TNG growth-response relationship and to determine the test response means against the control using Dunnett's procedure (Steel and Torrie 1960) and Williams's statistic t_k (Williams 1972). Williams's test has been developed specifically to test for the effect of set of increasing dosage type treatments. It assumes that the effect of increasing dose produces a steadily increasing positive or negative response. For data with equal replication at each challenge dose, it is the more sensitive test for differences between a dose level and the control. However, for these data, the test results were identical using Williams's and Dunnett's procedure.

	Lowest Significant Response Concentrations (mg/l)	
	N. pelliculosa	S. capricornutum
24-hour	1.8	Not analyzed
96-hour cell counts	0.32	1.0
96-hour chlorophyll a	0.32	0.1

These results suggest that S. capricornutum chlorophyll a values were significantly affected at the lowest dose tested. However, the influence of the varying amounts of lactose in this experiment is not known and makes this result questionable. N. pelliculosa was significantly affected at 0.32 mg/l but not at 0.1 mg/l.

Aquatic Invertebrates. Acute toxicity studies conducted by Bentley et al. (1978) demonstrated that TNG is toxic to invertebrates. Three species of macroinvertebrates and one zooplankton species were tested under static conditions in acute toxicity tests of 48 hours duration. These organisms were: the amphipod Gammarus fasciatus; the isopod Asellus militaris; the midge Chironomus tentans; and the cladoceran Daphnia magna. Forty-eight-hour flow-through tests were also conducted using Daphnia and Chironomus larvae. Based on 48 hours, the EC50 values for these four organisms ranged from 46 to 55 mg/l of TNG. The flow-through tests resulted in slightly lower 48-hour EC50s: 32 mg/l for Daphnia magna and 20 mg/l for Chironomus tentans and incipient EC50s of 25 and 18 mg/l, respectively.

Chronic studies, conducted over two generations, indicated that lower TNG levels produce effects. In Daphnia, production of young was significantly affected ($p < 0.05$) at 12.5 mg/l, but not at 6.2 mg/l. For Chironomus tentans, survival of second generation larvae was significantly affected at 3.1 mg/l but not at 1.5 mg/l.

Maximum acceptable toxicant concentration (MATC)* limits for Daphnia magna were therefore 6.2 to 12.5 mg/l. MATC limits for Chironomus tentans were found to be 1.5 to 3.1 mg/l. These data suggest that invertebrates as a group are less sensitive to TNG than fish during both short- and long-term exposure.

*Maximum Acceptable Toxicant Concentration (MATC): the highest concentration of toxicant that has no adverse effect on survival, growth or reproduction of a species based on the results of a life-cycle or partial life-cycle toxicity test. A life-cycle or partial life-cycle test cannot produce a value for the MATC; a test can only produce limits within which the MATC must fall

Fish. Based on the work of Bentley et al. (1978) and limited data from Hemphill (1975), fish appear to be the most sensitive group of aquatic organisms to TNG. Bentley et al. (1978) conducted static and flow-through acute bioassays of TNG using bluegill (Lepomis macrochirus), channel catfish (Ictalurus punctatus), and fathead minnow (Pimephales promelas) as test organisms. Static acute tests were also conducted using rainbow trout (Salmo gairdneri). Hemphill (1975) conducted static, acute bioassays of TNG, ethylene glycol dinitrate (EGDN) and a mixed waste containing both TNG and EGDN. The tests conducted by Bentley and Hemphill are not completely comparable in that Bentley used well water while Hemphill used a natural creek water. Chronic studies of the effects of TNG were conducted by Bentley et al. (1978) using fathead minnows and channel catfish as test organisms. These authors also performed a limited bioconcentration study for ^{14}C TNG in three fish species: P. promelas, I. punctatus, and S. gairdneri. Test procedures followed the most current EPA techniques (EPA 1975).

TNG exerts an acute toxic effect on fish at low concentrations. Table 5 presents the 96-hour LC50s found by Bentley et al. (1978) for both static and flow-through test procedures. The data base for static fish bioassays consists only of nominal concentrations; TNG concentration was monitored in the flow-through tests. The bluegill, used to test the effect of changes in temperature, pH and water hardness, was the most sensitive organism to the acute effects of TNG, having a minimum static 96-hour LC50 of 1.38 mg/l at 20°C, pH 6.0, and 35 mg/l of total hardness. The relatively small differences in 96-hour LC50 values noted in these tests indicate that the water quality variables tested have little effect on TNG toxicity. Flow-through test results did not differ from static, acute LC50s.

The acute toxicity to various life stages was examined under static conditions using the fathead minnow as the test organism. One-hour-old fry had a 96-hour LC50 of 5.5 ± 1.0 mg/l* and were the least sensitive age group tested. Egg hatchability (144-hour LC50 = 1.2 ± 0.7 mg/l*), however, was more susceptible to TNG toxicity than the other stages tested. These data imply that no one stage is much more sensitive than any other. Incipient LC50's for channel catfish, bluegill, and fathead minnow were 0.50 ± 0.14, 0.55 ± 0.13, and 1.87 ± 0.72 mg/l TNG,* respectively.

In general, the fathead minnow results discussed above are confirmed by Hemphill's (1975) work that also utilized nominal concentrations. In his study, pure TNG gave a 96-hour LC50 of 4.2 + 0.08 mg/l* at 20°C. He found that the LC50 for this compound was 23 + 4.6 mg/l at 10°C. LC50 for EGDN was 5.2 mg/l, essentially the same as for TNG. Bioassays of TNG production wastewater and TNG-EGDN mixed wastewater gave LC50 values above a total nitrate ester concentration of 10 mg/l.

The marked temperature dependence of the LC50 for TNG was not confirmed by Bentley et al. (1978) using the bluegill as the test organism. However, this latter author did not test temperature dependence of the LC50 to 10°C. The mean 96-hour LC50 for L. macrochirus at 15°C was 3.55 mg/l compared to 1.92 mg/l at 20°C for this organism. The LC50 for rainbow trout tested

*Mean and 95 percent confidence interval.

only at 10°C was in the same range as the 20°C LC50 for the other fish tested (Table 5).

Eight-day bioconcentration of the carbonaceous residue of TNG in fish muscle is low (8 to 15X) (Bentley et al. 1978). In this study, bluegill, catfish, trout and fathead minnow were continuously exposed to 0.42 ± 0.10 mg/l ^{14}C labeled TNG for eight days. None of the exposed organisms appeared to suffer any effects due to TNG and no significant increase in ^{14}C residues was observed after 4 days exposure. Bioconcentration potential of TNG therefore appears to be an unimportant factor in establishing water quality criteria.

The longer term tests carried out by Bentley et al. (1978) were analyzed by analysis of variance (ANOVA) followed by Dunnett's comparison of individual value with control. Unfortunately, a number of these analyses were made utilizing average values of the replicates within the various tests. In order to obtain the maximum amount of information from the data, the statistical analyses were repeated utilizing individual raw data values, pooling controls when appropriate and taking into account, when appropriate, the blocked experimental design. In several cases, this showed that lower levels of TNG were producing statistically significant effects than had been revealed previously. In addition to Dunnett's test, Williams's (1972) procedure was applied to the data. This test is specifically designed to detect differences in situations where an increasing or decreasing mean response with challenge concentrations is expected.

The longer-term test results on fish are summarized in Table 6. This table shows the lowest significant response concentrations.

In general, the longer-term tests showed significant effects at TNG concentrations an order of magnitude lower than the acute tests. Egg-fry studies were carried out by Bentley et al. (1978) on channel catfish and fathead minnows. These involved continuous exposure of eggs and fry for seven to thirty days. For channel catfish, effects were seen on both survival and length at 0.31 mg/l (no effect at 0.15 mg/l). Fathead minnows were slightly more sensitive with effects on survival observed in the third study at TNG concentrations as low as 0.06 mg/l (no effect at 0.03 mg/l). In the first (30-day) fathead minnow study, significant effects on length were seen at the lowest test concentration, 0.12 mg/l. In the third fathead minnow study, the length data did not respond in an orderly progression with increasing TNG concentration. This produced anomalous results using the Dunnett's procedures, i.e., significant differences at 0.03 mg/l, but no significant difference at 0.06 mg/l. Williams's procedure indicated significant differences at the lowest concentration, 0.03 mg/l. However, three factors suggest that this conclusion is not valid: 1) the anomalous nature of the data, 2) the statistical analysis showed unusual variation within replicates, and 3) the results were not confirmed in later life cycle tests.

A full life cycle study was carried out by Bentley et al. (1978). Beginning with fry, the test extended to 30-day-old second generation fish. Survival of first generation fish after 266 days exposure, and survival and

TABLE 5

ACUTE TOXICITY OF TNG FOR FOUR FRESHWATER FISH[a]

Fish Species	96-Hour LC50			
	Flow-Through Test[b]	Static Tests		
		Mean	Range	No. of Tests
Rainbow Trout *Salmo gairdneri*	-	2.8	-	1
Channel Catfish *Ictalurus punctatus*	>1.87	3.2	-	1
Fathead Minnow *Pimephales promelas*	3.0	3.1	2.1-5.5	4
Bluegill *Lepomis macrochirus*	1.67	1.97	1.38-3.55	15

[a]Reference: Bentley *et al* (1978).

[b]One test performed for this category.

TABLE 6

LONG-TERM TEST RESULTS FOR FISH

Test Description	Variable	Lowest Significant Response Concentrations	
		Dunnett	Williams
Egg-Fry Studies			
Channel Catfish - 30-day study	% Hatch	N.E.	N.E.
0.08 - 1.25 mg/l	% Survival	0.31	0.31
	Length	0.31	0.31
Fathead Minnow - 30-day study	% Hatch	0.47	0.47
0.12 - 1.87 mg/l	% Survival	0.12[a]	0.12[a]
	Length	0.12[a]	0.12[a]
Fathead Minnow - 7-day study	% Hatch	3.75	3.75
0.23 - 3.75 mg/l	% Survival	0.23[a]	0.23[a]
Fathead Minnow - 30-day study	% Hatch	0.47	0.47
0.03 - 0.47 mg/l	% Survival	0.12	0.06
	Length	A.R.	0.03[a]
Full Life Cycle Test			
Fathead Minnow	30-Day % Survival	A.R.	N.E.
0.11 - 1.75 mg/l	30-Day Length	A.R.	0.43
First Generation	60-Day % Survival	0.22	0.22
	60-Day Length	N.E.	N.E.
	174-Day % Survival	0.22	0.11[a]
	266-Day % Survival	0.22	0.22
	266-Day Male Length	0.43	0.43
	266-Day Female Length	N.E.[b]	N.E.[b]
	266-Day Male Weight	0.43	0.43
	266-Day Female Weight	N.E.[b]	N.E.[b]
Second Generation	% Hatch	0.43	0.43
	30-Day % Survival	0.22	0.22
	30-Day Length	0.22	0.22

N.E. = No observable effect.

A.R. = Anomalous results.

[a]Lowest concentration tested.

[b]Data only to 0.43 mg/l; all other fish dead.

length of second generation fish at the termination of the test were affected at TNG concentrations as low as 0.22 mg/l. Survival of first generation fish after 172 days exposure was significantly reduced at the lowest test concentration, i.e. 0.11 mg/l, according to Williams's procedure, although Dunnett's test, a somewhat less sensitive test, failed to indicate a significant affect at this level.

Mechanisms of Toxicity. There are no published reports on toxic mechanisms for TNG in aquatic organisms. Bioaccumulation factors for TNG carbon residues in fish are low; nevertheless TNG apparently is cumulatively toxic. This evidence for cumulative toxicity is derived from a comparison of acute and chronic lethal levels (Bentley et al. 1978). Male fish were found to be more sensitive to long-term exposure to TNG than females. Bentley et al. (1978) suggested that increased membrane permeability may be a means whereby TNG exerts toxicity. These authors suggested that changes in the permeability due to chronic exposure to TNG may increase long-term toxic effects compared to acute effects.

Some inferences can be made considering the metabolism of the compound in mammalian studies. Since certain biochemical pathways are fundamental to all living systems, an assumption can be made with a high degree of probability that selected toxic reactions observed in mammals also occur in aquatic organisms. In this regard, the TNG molecule could be initially metabolized by enzymatic cleavage liberating a nitrite group. Presence of NO_2^- within an organism will uncouple oxidative phosphorylation. This will result in a major interference with a principal metabolic pathway for energy transformation and electron transfer. Dacre and Tew (1973) reported that nitroglycerin poisoning in mammals presented a chemical pathway similar to that of sodium nitrite. On a weight basis, however, TNG was about twice as toxic as $NaNO_2$ to mammals.

Russo and Thurston (1977) have examined the toxicity of NO_2^- to fish and reported a 96-hour LC50 of 7.5 mg N/l to adult catfish (Ictalurus punctatus); 0.22 - 0.33 mg N/l for trout (Salmo gairdneri); and 2.35-3.81 mg N/l for the fathead minnow (Pimephales promelas). The toxic response in these organisms was reported to result from irreversible conversion of hemoglobin to methemoglobin by the NO_2^- ion. TNG reportedly causes methemoglobin in mammals (Ellis et al. 1978). Another way in which cleaved NO_2^- groups may elicit a toxic response is by combining with secondary amines to form nitrosamines, which have documented toxic properties (Dacre and Tew 1973). Based on percent nitrogen (14 percent), the acute toxicity data of Bentley et al. (1978) would have ranged from 0.18 to 0.77 mg N/l, 96-hour LC50. This is in the same range of acute toxicity due to the NO_2^- ion.

A significant portion of the toxic effect of TNG may stem from enzymatic liberation of NO_2^- intracellularly. The effects of carbonaceous transformation products have been unexplored. In rats, TNG reportedly causes hepatic carcinoma (Ellis et al. 1978) when given in oral dosages. No similar pathological or metabolic studies have been done on aquatic organisms.

IV. Environmental Fate and Effects

Nitroglycerin wastes have been released to the aquatic environment at BAAP and RAAP. Both of these are U.S. Army facilities operated by private concerns under contract to the Army. Lengthy field efforts were conducted both at the Badger plant in Wisconsin and the Radford facility in Virginia. The objective of these investigations was to examine the impact of TNG on selected indigenous biota in the receiving waters. In both of these studies, macroinvertebrates and algae were the foci of investigation. Selection of these communities was made because of their short response time to environmental stresses. The limited mobility of these attached and burrowing forms also serves to locate an impact zone. Examination of artificial substrates placed in the receiving water was beneficial to the studies since the extent of colonization could be determined within and away from the impact zone.

Battelle Columbus Laboratories conducted the survey at the Badger plant where two on-site ponds receive TNG waste products (Stilwell et al. 1976). One of these ponds received wastewater from the synthesis of TNG and the other from the manufacture of double-based solid propellant (rocket paste) made up of TNG plus nitrocellulose. The ponds were characterized in terms of concentrations of munitions and other standard water quality parameters. Sediments were also collected and analyzed for TNG and parameters such as chemical oxygen demand and total Kjeldahl nitrogen.

Biological assessments at BAAP were made on the phytoplankton, periphyton, and macroinvertebrates. Observations were completed on natural communities and those colonizing artificial substrates. The results of surveys carried out in 1974 and 1975 were presented in two volumes (Stilwell et al. 1976). Stilwell concluded from an extensive array of chemical and biological analyses that TNG wastes did have an impact on those communities examined and recommended a "no-effect" threshold for TNG of <3 mg/l. This level was based on the condition of the phytoplankton, periphyton, and macroinvertebrate communities related to observed TNG concentrations.

A review of the data base at BAAP (Stilwell et al. 1976) showed that the responses observed in the biotic community were the product of a number of environmental factors. The pond receiving TNG production wastewater (nitroglycerin pond) was also stressed by high dissolved-solids content (5 to 6 times background), nitrate (up to 205 ppm NO_3), and low pH (3.1-3.5). The nitroglycerin pond sediments contained concentrations of 31 to 43 mg/kg TNG. Otherwise sediment quality in this pond appeared to be unaffected.

The pond receiving propellant-manufacturing wastewaters (rocket paste pond) represented a more restrictive environment. This pond was characterized by low oxygen concentrations and anaerobic sediments which contained high concentrations of chemical oxygen demand and Kjeldahl nitrogen. TNG concentrations in these sediments ranged from <2 to 2.2 mg/kg. In the water column, TNG concentrations ranged from 4.8 to 9.0 mg/l in the nitroglycerin pond and <0.6 to 3.9 mg/l in the rocket paste pond.

Examination of the algal populations found in these ponds revealed conditions suggesting environmental stress. The phytoplankton community in the nitroglycerin pond was dominated by two species of Scenedesmus, one of which has been considered to be extremely pollution-tolerant (Palmer 1969). Eighteen percent of the standing phytoplankton crop consisted of two cyanophyte species. A total of only six species comprised the community. In the rocket paste pond, only nine species were found and cyanophytes dominated the population. The artificial substrates were dominated by cyanophytes and periphyton community diversity was low in both receiving waters. The autotrophic community colonizing the artificial substrates was much reduced compared to heterotrophs and, in general, chlorophyll a levels were less than 1 mg/m^3 on these substrates.

Benthic macroinvertebrates were absent in pond sediments. Hester-Dendy artificial substrate samplers were not colonized after incubation periods up to five weeks. Causal factors probably include low pH and anaerobic sediments as well as direct toxicity from TNG wastes.

Poor environmental quality as reflected by the condition of the indigenous biotic communities at BAAP resulted from several types of environmental stress. Much of this stress was a result of discharges of waste products of nitroglycerin manufacturing, i.e., NO_3-N, SO_4, organic material, waste acids, etc. TNG concentrations undoubtedly played a role but could not be considered as the only impact. Therefore, assigning a direct cause and effect relationship of adverse environmental response to the observed levels of TNG is questionable.

The concentrations found in pond sediments, however, may have had a direct role in excluding invertebrates from these environments. The data of Bentley et al. (1978) discussed in Section III imply that severe acute effects on macroinvertebrates might be expected at the concentrations observed in the nitroglycerin pond (i.e., 30-40 ppm). Bentley also reported MATC limits for one chironomid of 1.5 to 3.0 mg/l TNG in water. Similar levels of TNG were present in the sediments of the rocket paste pond. Other conditions in the rocket paste pond suggest that a chironomid fauna might occur there.

All production of munitions was halted at BAAP in May 1975, when the plant went into standby status on instructions from U.S. Army Armament Command. Since this occurred prior to the intensive sampling program, no firm inference can be drawn relating to the persistence of TNG in receiving waters. The waters of the nitroglycerin pond contained 4 to 6 times as much TNG as the pond influent. In the rocket paste pond, concentrations ranged up to half the concentrations found in a single composite effluent sample. These data suggest, however, that low levels of TNG may persist in the water column. More importantly, the results show that TNG finds its way into aquatic sediments and may build up to environmentally significant concentrations.

Field studies were carried out at RAAP by Wapora, Inc. (Huff et al. 1975). The RAAP facility, currently the only active military producer of TNG, discharges munitions production wastewaters into the New River near Radford, Virginia. Rosenblatt et al. (1973) predicted potential TNG concentrations resulting from 33 lbs/day discharge from RAAP and a set of "worst

case" river flow conditions. Assuming no degradation, TNG concentrations would reach 12 µg/l (0.012 mg/l) 30 miles downstream and 0.8 µg/l at the point where the diluted discharge meets the Ohio River, approximately 80 miles from RAAP.

Huff et al.(1975) examined water and sediment quality and populations of macroinvertebrates, fish and periphyton in the New River below the nitroglycerin waste discharge points during a four-day field study. The short duration of the study, small number of samples, and the long length of the river reach surveyed preclude anything but the most general conclusions concerning the impacts on the receiving system. Effluent, water, and sediment TNG levels were not quantitated. The authors were therefore unable to assign any causal relationship of TNG to observed impacts on macroinvertebrates and fish communities. In general, the major impact occurred due to discharges from other portions of the facility located downstream of the TNG production area.

In an attempt to focus more closely on the impact of TNG wastewaters, Weitzel et al. (1976) surveyed a 1.4-km reach of the New River. Collections were made during May-June and again in October-November. Three stations were located in the reach receiving TNG wastewaters. Two reference stations were located above the TNG production outfalls, and one station was located 900 m downstream of the impact area in order to observe recovery.

Analysis was made of the water and sediment chemistry, industrial effluent quality, and the periphyton and macroinvertebrates colonizing natural and artificial substrates. The results showed the presence of TNG in the water at all stations, including the control station, generally at concentrations of 0.01 to 0.02 mg/l. Nitroglycerin was found in only three of 32 sediment cores analyzed. The maximum amount in sediment was 1.5 mg/kg, which was observed 900 meters downriver from the outfalls in the recovery zone.

The aqueous environmental chemistry suggested that the dominant influence in the study area was derived from discharges from other parts of the facility rather than from the TNG production area. The primary impact from the TNG production area was an increase in o-nitrodiphenylamine (NDPA) and elevated lead levels found in the sediments below one outfall. Low levels of NDPA were also found in the sediments at the most downstream station along with the low level of TNG.

Weitzel et al. (1976) observed localized shifts in both the periphyton and macroinvertebrate communities. Localized inhibition of periphyton production was observed near one outfall but was not attributable to discharges from the TNG production area. Biostimulation at one impact station was attributed to increased nitrogen levels. None of the effects observed could be related to TNG discharge concentrations, therefore, the authors were unable to estimate a no-effect level.

During the study, discharge concentrations of TNG were generally about one-tenth those used by Rosenblatt et al. (1973) to predict downstream maximum concentrations. The low discharge volumes with respect to river flow

tend to preclude determining any environmental effects due to TNG. Discharges of other pollutants from TNG production along with TNG itself as well as stresses resulting from activities in other parts of the RAAP facility were instrumental in masking any possible TNG effect. Additionally, river flow fluctuates over an order of magnitude daily in the survey reach due to water released from a hydroelectric dam upstream. This fluctuation created a very significant hydraulic stress on aquatic communities inhabiting the study area.

Studies of TNG environmental fate at both BAAP and RAAP showed that TNG discharged into a receiving water deposits to some degree in aquatic sediments. In one environment sampled at BAAP, relatively high concentrations were encountered. At RAAP, the scouring action of the fluctuating river flow would tend to prevent sediment deposition.

Nothing is known about the half-life or secondary breakdown products of TNG that might be formed in the environment. None of the field studies considered this aspect and no conclusion can be made regarding TNG transformations in the aquatic ecosystem. An associated compound, nitrodiphenylamine, used to stabilize TNG, was detectable in the New River. No analyses were made at BAAP for this stabilizer. The aromatic structure of this secondary amine, however, would suggest a toxic mode of action to aquatic biota.

Studies of methods of treatment of TNG indicate that products of microbial and chemical hydrolysis are formed which could evoke a toxic response. This is especially probable of glycidyl nitrate or glycidol. Both of these compounds contain epoxy groupings which could act in an inhibitory manner in an aquatic system.

In summary, field studies indicate biological effects in waters receiving TNG manufacturing wastes. Other natural variables or those associated with TNG production masked discrete cause and effect relationships. Macroinvertebrate communities were completely nonexistent in the presence of sediment TNG concentrations of up to 2.2 mg/kg in one environment and up to 43 mg/kg in a second.

V. Criteria Development

In developing water quality criteria, all available data were considered. However, the laboratory bioassay results were the only data where TNG concentrations could be related directly to biologic effects. Three approaches outlined below were compared in order to arrive at suitable criteria:

1. The proposed EPA procedure as outlined in the Federal Register (EPA 1978).

2. Traditional approaches (APHA 1975; NAS 1973; EPA 1976) which develop criteria based on application factors and acute toxicity values (EC or LC50's) for sensitive species.

a. The lowest LC or EC50 value found multiplied by a conservative application factor. This factor is chosen by experience in order to provide a conversion to chronic effects based on the nature of the toxicant.
b. The lowest LC or EC50 value found multiplied by the lowest experimentally derived application factor.

Proposed EPA Procedure. The recently proposed EPA procedure provides a very detailed protocol for evaluating a bioassay data base to determine water quality criteria. Precise procedures have been described for converting data to a common basis and for deriving criteria from the converted data. This procedure may or may not be adopted in its present form. To facilitate the reader's understanding of this procedure, the same paragraph notation will be utilized herein as is used in the "Guidelines" section of the EPA procedure. All data are for freshwater.

I. Final Fish Acute Value
A. Data base (see Tables 7 and 8).
B. Calculate LC50 values converted to measured concentrations (see Tables 7 and 8).
C. All results are already on 96-hour basis; therefore, no conversion is required.
D. Calculate LC50 values converted to flow-through tests - (see Table 7).
E. For each species the geometric mean of the LC50 values is shown in Table 9.
F. The geometric mean of all species geometric means is 1.6 mg/l.
G. The final fish acute value is the lower of the following values:
1. The geometric mean from F, above, divided by 3.9, i.e. 0.41 mg/l.
2. The lowest LC50 value (corrected when necessary) based on measured concentrations from a flow-through test, i.e., 0.75 mg/l.
Therefore, the final fish acute value for TNG is 0.41 mg/l.

II. Final Fish Chronic Value
A. Calculate chronic values as the geometric mean of MATC limits for life cycle or partial life cycle tests. Use one-half of the geometric mean if the result has been derived from an embryo-larvae test (see Table 10 for results).
B. Acceptable chronic values are available.
C. The final fish chronic value is the lowest of the following values:
1. The lowest individual chronic value which is 0.021 mg/l.
2a. For each species the geometric mean of the geometric means for that species is shown in Table 10.
b. The geometric mean of geometric means for all species is 0.048 mg/l.
c. Divide this mean by 6.7 to obtain a value of 0.0072 mg/l.
3. This item requires matched pairs of MATC limits and 96-hour LC50 values based on flow-through tests with measured concentrations. Since all 96-hour LC50 testing was done using nominal or calculated concentrations, no such matched pairs exist.

TABLE 7

DATA BASE FOR FISH ACUTE VALUE
TNG - STATIC, 96-HOUR LC50s

Static Test - Calculated on Nominal Concentrations	LC50 Values at 96 Hours, mg/l
Bluegill	2.7, 3.55, 1.92, 1.99, 1.76, 1.51, 1.65, 1.38, 1.91, 2.1, 2.0, 2.0, 2.0, 1.7, 2.1
Fathead Minnow	2.5, 5.5, 2.1, 2.1, 3.4, 4.2[a], 23.5[a]
Trout	2.8
Catfish	3.2
Multiply by 0.77 to simulate results based on measured concentrations.	
Bluegill	2.08, 2.73, 1.48, 1.53, 1.36, 1.16, 1.27, 1.06, 1.47, 1.62, 1.54, 1.54, 1.54, 1.31, 1.62
Fathead Minnow	1.93, 4.24, 1.62, 1.62, 2.62, 3.23, 18.1
Trout	2.16
Catfish	2.46
Multiply by 0.71 to simulate results based on flow-through tests.	
Bluegill	1.48, 1.94, 1.05, 1.09, 0.96, 0.83, 0.90, 0.75, 1.04, 1.15, 1.09, 1.09, 1.09, 0.93, 1.15
Fathead Minnow	1.37, 3.01, 1.15, 1.15, 1.86, 2.30, 12.8
Trout	1.53
Catfish	1.75

[a] These two values from Hemphill 1975. All other data from Bentley et al. 1978.

TABLE 8

DATA BASE FOR FISH ACUTE VALUE
TNG - FLOW-THROUGH, 96-HOUR LC50s

Calculated on Nominal Concentrations	LC50 Values at 96 Hours, mg/l
Bluegill	1.67
Fathead Minnow	3.0
Catfish	>1.87
Multiply by 0.77 to simulate results based on measured concentrations.	
Bluegill	1.29
Fathead Minnow	2.31
Catfish	>1.44

All data from Bentley et al. 1978.

TABLE 9

COMBINED DATA BASE FOR FISH ACUTE VALUE
TNG - 96-HOUR LC50s

	Geometric Mean of All LC50 Values by Species, mg/l
Bluegill	1.09
Fathead Minnow	2.26
Trout	1.53
Catfish	1.75

Geometric mean of species geometric means - 1.60 mg/l.

TABLE 10

DATA BASE FOR FISH CHRONIC VALUE
TNG

Life-Cycle Tests - Concentration in mg/l			
	MATC Limits	Geometric Mean	
Fathead Minnow	0.0-0.11	<0.11	
Egg-Fry Tests	**MATC Limits**	**Geometric Mean**	**1/2 Geometric Mean**
Fathead Minnow	0.03-0.06	0.042	0.021
Catfish	0.15-0.31	0.22	0.11
Species Geometric Mean			
Fathead Minnow - 0.021 mg/l			
Catfish - 0.11 mg/l			
Overall Geometric Mean - 0.048 mg/l			

All data from Bentley et al. 1978.

Therefore, the final fish chronic value for TNG is 0.0072 mg/l.

III. Final Invertebrate Acute Value
- A. Invertebrate data (EC50 at 48 hours) are shown in Table 11.
- B. Acceptable data are available for invertebrates.
- C. Calculate EC50 values converted to measured concentrations; (see Table 11).
- D. EPA protocol contains no item III-D. However, W. A. Brungs, Technical Assistance Director, EPA Environmental Research Laboratory, Duluth, Minn. has indicated a correction factor of 0.43 should be applied to convert 48-hour EC50 values to 96-hour EC50 values (see Table 11).*
- E. Calculate EC50 values converted to flow-through (see Table 11).
- F. For all species calculate the geometric mean of all EC50 values.
- G. Geometric mean of all species geometric means is 15 mg/l.
- H. The final invertebrate acute value is the lower of the following values:
  1. The geometric mean from G, above, divided by 21, i.e. 0.71 mg/l.
  2. The lowest EC50 value based on measured concentrations from a flow-through test.

Therefore, the final invertebrate acute value for TNG is 0.71 mg/l.

IV. Final Invertebrate Chronic Value
- A. Chronic values calculated as the geometric mean of the MATC limits from life-cycle or partial life-cycle tests (see Table 12).
- B. Acceptable chronic values are available.
- C. The final invertebrate chronic value is the lower of the following values:
  1. The lowest chronic value, i.e., 2.2 mg/l.
  2. The geometric mean of the geometric means for all species divided by 5.1, i.e., 4.4/5.1 = 0.86 mg/l.

Therefore the final invertebrate chronic value is 0.86 mg/l.

V. Final Plant Value
- A. Tests were conducted on four species of algae.
- B. The lowest concentrations tested affected Selenastrum capricornutum. However, faulty control of this experiment invalidates the results. Therefore, the lowest available plant toxicity value is the "acute" (i.e. 96-hour) no-effect level based on growth inhibition of Navicula pelliculosa, i.e. >0.1<0.32 mg/l, geometric mean = 0.18 mg/l.

Therefore, the final plant value for TNG is 0.18 mg/l.

VI. Residue Limited Toxicant Concentration (RLTC)
No data exist for this analysis.

*Personal Communication, 1978.

TABLE 11

DATA BASE FOR ACUTE INVERTEBRATE VALUE
TNG - 96-HOUR LC50s

Nominal or calculated concentrations	Static Test EC50 at 48 Hours mg/l	Flow-Through EC50 at 48 Hours mg/l
Water Flea	46	32
Scud	50	--
Sowbug	50	--
Midge	55	20
Multiply by 0.77 to simulate results based on measured concentrations		
	EC50, mg/l	EC50, mg/l
Water Flea	35	25
Scud	39	--
Sowbug	39	--
Midge	42	15
Multiply by 0.43 to simulate results based on 96-hour tests		
	EC50, mg/l	EC50, mg/l
Water Flea	15	11
Scud	17	--
Sowbug	17	--
Midge	18	6.5
Multiply by 1.1 to simulate results based on flow-through results		
	EC50, mg/l	EC50, mg/l
Water Flea	17	11
Scud	18	--
Sowbug	18	--
Midge	20	6.5
Species Geometric Means		
	EC50, mg/l	
Water Flea	14	
Scud	18	
Sowbug	18	
Midge	11	

TABLE 12

DATA BASE FOR INVERTEBRATE CHRONIC VALUE

Life_cycle test. Concentrations nominal or calculated.

	MATC Limits	Geometric Mean
Water Flea	6.2 - 12.5	8.8
Midge	1.5 - 3.1	2.2

All data from Bentley et al. (1978).

VII. Other Data
Other data will be considered in other criteria setting procedures which follow.

VIII. Final Values
A. The final acute value is the lower of the final fish acute value and the final invertebrate acute value, i.e., 0.41 mg/l.
B. The final chronic value is the lowest of 1) the final fish chronic value, 2) the final invertebrate chronic value, 3) the final plant value, and 4) the RLTC, i.e., 0.0072 mg/l.

IX. Criterion
A. Sufficient data are available to establish a criterion for TNG under the guidelines
B. The maximum TNG concentration should never exceed the final acute value of 0.41 mg/l.
The 24-hour average TNG concentration should never exceed the lower of the following two values:
1. The final acute value from item VIIIA, above, times 0.44, i.e. (0.41) (0.44) = 0.18 mg/l.
2. The final freshwater chronic value from item VIIIB, above, i.e. 0.0072 mg/l.
Therefore, the 24-hour average TNG concentration should not exceed 0.0072 mg/l.

Traditional Approach - Traditionally, environmentally safe concentrations have been derived when only acute toxicity data are available by use of an application factor. This factor is applied to the lowest acute (96-hour LC50) toxicity value. A general factor may be utilized or a factor may be experimentally derived.

1. General Application Factor - Where the material in question is nonpersistent and believed to have noncumulative effects a 0.05 application factor is utilized to determine the 24-hour average value. For toxicants which are persistent or cumulative an application factor of 0.01 is recommended (NAS 1973; EPA 1976A). The dearth of evidence documenting either the rate of transformation of TNG in aquatic systems or the persistence of its degradation products would indicate that the more conservative application factor of 0.01 is appropriate for TNG. Applying this factor to the lowest acute toxicity result, 1.38 mg/l for bluegill, gives a safe concentration of 0.014 mg/l. This result is twice that obtained utilizing the recent EPA procedure.

2. Experimentally Derived Application Factor - A third procedure for deriving environmentally safe concentrations is similar to the previously discussed procedure except that the application factor is experimentally derived. Where both acute and chronic data are available, the application factor is the ratio of the lower MATC limit to the lowest EC50 or LC50 for that species.

If data for more than one species exist, the minimum application factor will be utilized. This factor is then multiplied by the lowest LC50 or EC50 value for any species to determine an environmentally safe concentration. The experimental application factors are shown in Table 13. Utilizing the lowest application factor of 0.014 and the lowest 96-hour LC50 for bluegill of 1.38 mg/l, a value of 0.019 mg/l is obtained.

The three procedures give the following results:

Procedure	Water Quality Criteria 24-hour Average
Proposed EPA	0.0072 mg/l
General Application Factor	0.014 mg/l
Experimentally Derived Application Factor	0.019 mg/l

From the chronic toxicity tests the most sensitive response was obtained on a 30 day egg-fry study using fathead minnows. Percent survival was reduced at a concentration of 0.06 mg/l and no significant effect was noted at 0.03 mg/l. The lowest measured no-effect concentration of 0.03 mg/l is 1.5 to 4 times higher than the criteria calculated above. Furthermore, the criteria are all specified as 24-hour average values, whereas the chronic test results are based on weeks or greater exposure time. Thus, while fathead minnows may not be the most sensitive species, there seems to be a reasonable safety factor between the measured no-effect concentration for one species and a general criterion.

Considering the information available at this time it is recommended that the water quality criteria for TNG of 0.01 mg/l as a 24-hour average. This should adequately protect aquatic organisms.

TABLE 13

EXPERIMENTALLY DERIVED APPLICATION FACTORS - TNG

Species	Lowest Reported Acute Value		Chronic Value		Application Factor
	Type of Reported EC50/LC50	EC50/LC50 (mg/l)	Response Parameter(s)	MATC Limits (mg/l)	
Channel Catfish	Nominal, 96-hr. LC50, Static	3.2	Egg-Fry - 30-day Test; Survival, Length	0.15-0.31	0.047
Fathead Minnow	Nominal, 96-hr. LC50, Static	2.1	Egg-Fry - 30-day Test; Survival	0.03-0.06	0.014
			Full Life-Cycle Test; 1st Generation - 174 day Survival	<0.11	<0.052
Daphnia	Nominal, 48-hr. EC50, Flow-Thru	32	Full Life-Cycle Test; 2nd Generation - Number Offspring	6.2-12.5	0.19
Midge	Nominal, 48-hr. EC50, Flow-Thru	20	Full Life-Cycle Test; 2nd Generation - 13-day Larvae Survival	1.5-3.1	0.075

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